Electrical Self-Stimulation of the Brain: A Model for the Behavioral Evaluation of Toxic Agents

by Zoltan Annau*

Rats implanted chronically with electrodes in the posterior lateral hypothalamus were trained to press levers in order to stimulate the brain electrically. Brief exposures to low oxygen concentrations reduced the lever pressing rate proportionately with the reduction in inspired oxygen. Similar reductions in self-stimulation rates could be observed in animals exposed to carbon monoxide or the organic solvent, trichloroethylene. Prolonged exposures of animals to hypoxia in chambers where self-stimulation rates as well as food and water intake via lever pressing were monitored, indicated that as oxygen concentration declined self-stimulation rates showed a marked increase for 12 hr followed by a decline. Food and water intake were depressed. This increase in self-stimulation was only observed at low (20°C) ambient temperatures and was accompanied by central depletion of norepinephrine. At high (30°C) ambient temperatures, self-stimulation was depressed by hypoxia. The data show the importance of comparing acute with chronic exposure to toxic agents, as well as the influence of environmental temperature in influencing behavioral events. In addition, the data indicate that the self-stimulation technique offers unique advantages over behavior maintained by food or water reinforcers in evaluating toxic compounds.

The behavioral investigation of the effects of neurotoxic substances has been carried out in many laboratories by a diversity of techniques. These techniques have generally been the same as those used by experimental psychologists for many years, consisting mainly of positive or negative reinforcement schedules with either food or water serving as the positive reinforcer and electric shock to the feet as the negative reinforcer. One approach that has been rarely used is electrical self-stimulation of the brain. This is a technique that we have used in our laboratory rather successfully in the evaluation of certain toxic substances, and I will now describe some of the parameters that are important in its use.

Olds was the first investigator to find that electrical stimulation of certain regions in the brain has reinforcing consequences for animals (1). Starting with an initial experiment in which electrodes were placed in the septal area, Olds mapped large portions of the rat brain for placements where electrical stimulation had reinforcing consequences. He found that electrical stimulation of certain pathways leading from the brainstem to the forebrain, coarsing primarily through the lateral hypothalamus and medial forebrain bundle formed the positive reinforcement system (2). Animals with electrodes in these locations could be trained rapidly to press a lever in order to obtain electrical stimulation. This work aroused a great deal of interest amongst neuroscientists since many of the points of stimulation that Olds described were located in areas that seemed to be important in controlling the so-called primary drives, i.e., hunger, thirst and sexual behavior. The question that arose immediately was whether stimulation of these areas with electrodes was a way of stimulating hedonistic centers in the brain that were always involved when reinforcing events occurred in the animal's environment. While this debate has not been answered to everybody's satisfaction, it appears from many studies that under the right conditions the reinforcing properties of electrical brain stimulation are not very different, if different at all, from the reinforcing properties of primary reinforcers such as food and water. Subsequent behavioral, physiological and neurochemical studies have revealed that the pathway described by Olds and others coincided with the existence of certain neurochemical transmitter sub-

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stances in these pathways (3). Through the work of the Swedish histochemists it has become evident that whenever electrodes yield self-stimulation behavior, the electrodes are placed in areas that are either dipaminergic or noradrenergic (4). Thus it appears that at least the catecholamines are intimately involved in the regulation of this behavior. More direct evidence for the involvement of the catecholamines in the self-stimulation behavior comes from a long series of pharmacological studies initiated by Stein and colleagues (5–7). These studies have generally shown that drugs such as the amphetamines that release catecholamines from presynaptic terminals, will enhance the rate of self-stimulation behavior. Drugs that not only release but, in fact, deplete the brain of catecholamines, such as reserpine, will lead to a cessation of self-stimulation behavior. Without going into any of the details of the intriguing question as to which particular amine is modulating this behavior, we can say that the evidence accumulating from studies carried out by Breeze and co-workers as well as others, suggest that dopamine is involved (8, 9). This issue has not been settled and those who attribute an equal role to norepinephrine also add experimental evidence on a steady basis (10).

Since it is assumed that there is some kind of a balance between cholinergic and aminergic mechanisms in the brain in maintaining normal behavior, studies dealing with the cholinergic side of this question have also been carried out. Thus it has been demonstrated that agents that inhibit cholinesterase decrease self-stimulation rates (11, 12). This effect, however, can be blocked by agents such as scopolamine and atropine (13). Scopolamine in low doses has been shown to facilitate self-stimulation (14), and facilitation has also been observed by the cholinergic agonist, nicotine (15). Thus from these studies, it will appear that self-stimulation behavior is controlled by both major neurotransmitter systems in the brain and that an imbalance in either of them can lead to enhancement or depression of this behavior.

In our laboratory we have been using the self-stimulation technique for the last 10 years investigating a variety of environmental conditions. In our experimental paradigm, male hooded rats weighing approximately 350 g are chronically implanted with stainless steel electrodes in the posterior lateral hypothalamus. Following recovery, the animals are trained to press a lever in order to obtain a quarter-second duration pulsetrain of 60 cycle current from a constant current source. When sufficiently stable baselines have been obtained, the animals are exposed to the various experimental conditions.

In the first series of studies we were interested in the relationship between the rate of self-stimulation and the oxygen concentration of the inspired gas. Our interest was in the relationship between the oxygen availability in the central nervous system and the rate of on-going behavior as a possible predictor for toxic exposures where interference with oxygen delivery might occur. We also were interested in the effects of altitude upon the ability of organisms to function normally. Animals were exposed to various low-oxygen environments for 16-min periods and changes in self-stimulation rates during exposure were compared with rates before and after exposure (16). Our results, as shown in Figure 1, were clear-cut and quantifiable. As oxygen concentration decreased the rate of lever pressing also declined. In order to determine whether self-stimulation rate was a determining factor in the effects of hypoxia, we exposed animals to the same oxygen concentrations as before but lever pressing at approximately 50% of the rate. The reduction in response rate was accomplished by reducing the intensity of the stimulating current. The results seen in Figure 2 indicate that the animals working at the lower rate seemed to be much more sensitive to hypoxia in that even the mild hypoxic condition, such as 14% oxygen, depressed their behavior.

In order to control for this rate effect, we decided in a subsequent experiment to determine whether animals prepared with electrodes at loci that normally do not support rapid responding, would also be very susceptible to hypoxia. We compared, therefore, the effects of different oxygen concen-
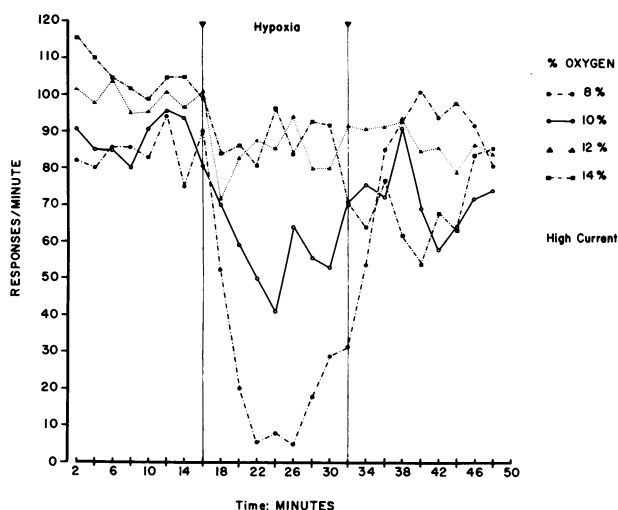

FIGURE 1. Mean self-stimulation rates of four rats exposed successively to 21% oxygen for 16 min to 8, 10, 12, or 14% oxygen for the next 16 min, and to 21% oxygen for the last 16 min. Each animal was exposed to all oxygen concentrations.
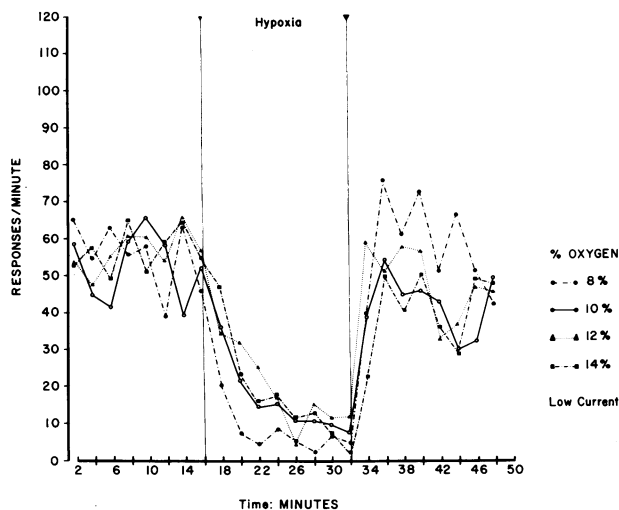
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FIGURE 2. Mean self-stimulation rates of four responding at low rates in 21% oxygen, (16 min). 8, 10, 12, or 14% oxygen (next 16 min), and in 21% oxygen (last 16 min). Each animal was exposed to all low oxygen concentrations.
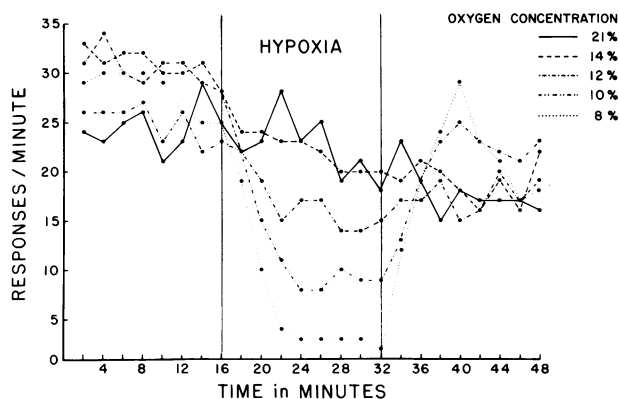

FIGURE 3. Mean self-stimulation rate of seven animals implanted with electrodes in the septal area exposed to 21% oxygen for 16 min, followed by a 16 min exposure to hypoxia and a 16-min recovery period in 21% oxygen.

trations on the rate of septal self-stimulation. Since septal animals respond at low rates and, therefore, the number of reinforcements per unit time decreased, we compared them with a group of hypothalamic animals that were trained on a FR-5 schedule (i.e., only every fifth response was reinforced) where we could maintain high rates but low density of reinforcement (17). It can be seen from Figure 3 that the low rate septal animals were as resistant to low oxygen as the rapidly lever pressing hypothalamic animals (in our previous study) and the animals on the FR-5 schedule (Fig. 4). Thus it would appear that the locus of electrical brain stimulation may be an important parameter in the evelution of toxic compounds.

To compare our effects during hypoxia with the effects of carbon monoxide, we exposed animals to various concentrations of carbon monoxide during self-stimulation sessions similar to those described above. Since carboxyhemoglobin levels of animals exposed to carbon monoxide do not reach equilibrium rapidly, these experiments could not be carried out in a meaningful manner during the short exposures that we had used with hypoxia. Figure 5 illustrates one such example of an animal exposed to 1000 ppm carbon monoxide. As carboxyhemoglobin levels rose the self-stimulation rate of the animal declined. In order to overcome the slowly changing physiologic condition of the animals, we pre-exposed them to the various carbon monoxide concentrations for 90 min and subsequently tested them during 1 hr self-stimulation sessions (18). As can be seen in Figure 6, there was a decrement in mean self-stimulation rate that became significant at 500 ppm and was highly significant at 1000 ppm during our experimental exposure. These data seem to be in general agreement with previous results using operant techniques in that the behavioral thresholds seem to be around 350–500 ppm (19–21). It might be worth noting that while the human data are not very consistent on the behavioral effects of carbon monoxide, Stewart's results some years ago also indicated that response decrements were seen at 500 ppm CO (22).
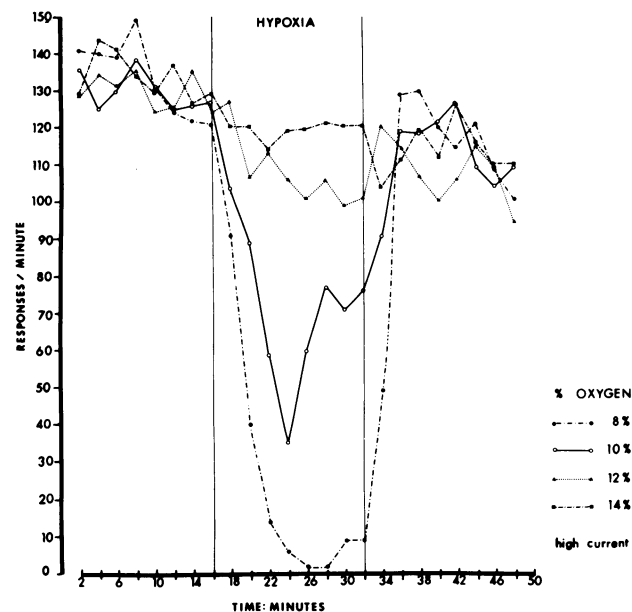

FIGURE 4. Mean self-stimulation rate of three animals implanted with electrodes in the posterior lateral hypothalamus and trained to self-stimulate on a FR-5 reinforcement schedule. As before, the first 16-min period of the experimental session was in 21% oxygen, the next 16 min in one of the hypoxic mixtures, and the last 16 min in 21% oxygen.
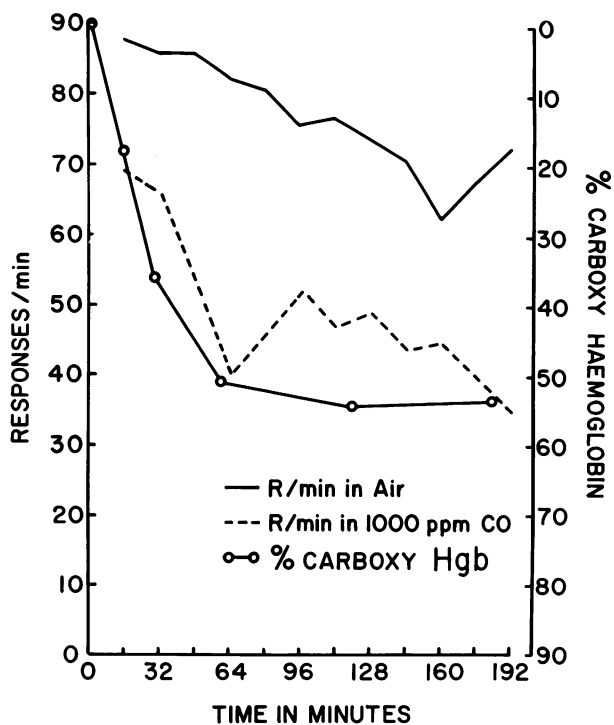
FIGURE 5. Comparison of the response rate of an animal during a 3-hr test session in air or 1000 ppm carbon monoxide in air. Carboxyhemoglobin values obtained from another animal with implanted arterial catheter are also shown.
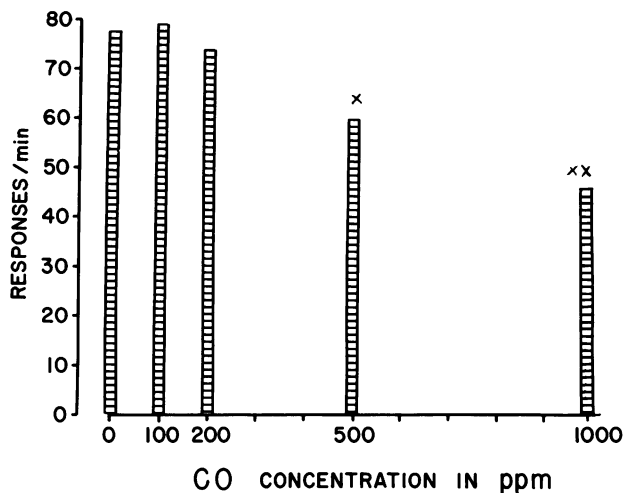

FIGURE 6. Mean self-stimulation rates obtained from a 1-hr exposure to various carbon monoxide mixtures. The self-stimulation test followed a 90-min passive exposure to the carbon monoxide concentration. The asterisk (*) denotes $p < 0.05$; the double asterisk (**) denotes $p < 0.01$.

In order to determine whether other toxic agents could also be used with the self-stimulation technique, we exposed animals to an organic solvent, trichloroethylene, during a series of experiments

(23). We compared animals before, during and after exposure to this agent in the normal condition as well as animals that were dehydrated. This condition was added since previous experiments had shown that animals could be made more susceptible to certain environmental poisons, such as antimony, by dehydration (24). Animals with electrodes in the lateral hypothalamus were exposed to two concentrations of trichloroethylene, 2500 ppm or 3000 ppm during 30-min test periods. Mean lever pressing rates obtained during the three days of control were compared with lever pressing rates during three consecutive days of 30-min exposures and the animals were subsequently followed for three days of recovery. Figure 7 indicates that the lower concentration of trichloroethylene exposure resulted in a significant depression in self-stimulation rates during the first day, particularly in the nondehydrated animals. During the second and third days of exposure there was considerable recovery in these animals so that they responded at approximately the same rate during the early stages of exposures as control animals. In the dehydrated animals (Fig. 8) on days 2 and 3 of exposure, there seemed to be an elevation of rate above control during the first 10 min of exposures, and this was followed by a decrease in rates to approximately the
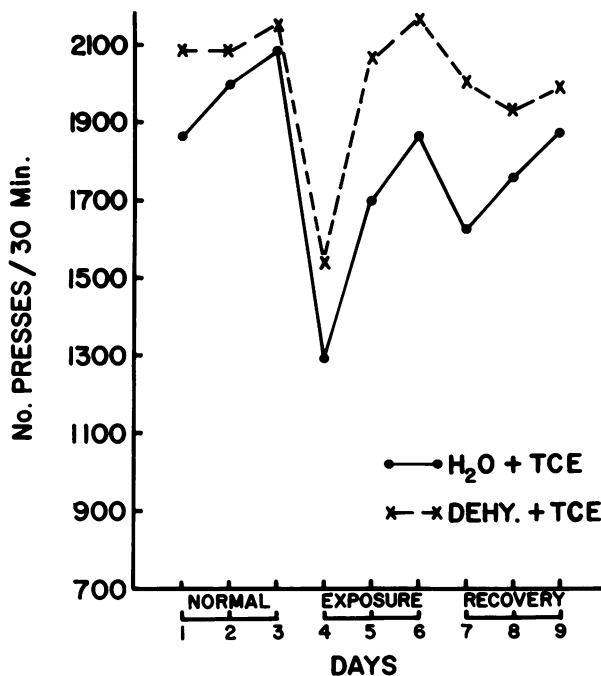

FIGURE 7. Mean self-stimulation rates of rats before, during and following exposure to 2500 ppm trichloroethylene. Duration of exposures was 30 min. The dehydrated group was water deprived during the three days of trichloroethylene exposure.
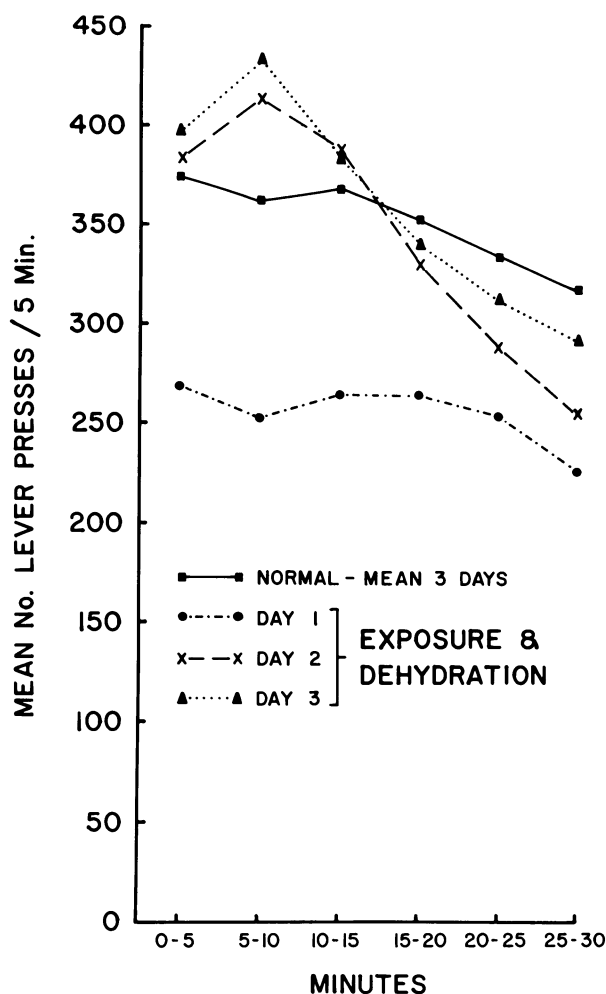
FIGURE 8. Response rate of rats as a function of 5-min interval before and during exposure of 2500 ppm trichloroethylene.

In order to be able to study longer periods of exposure to toxic agents, we modified our experimental procedure so that animals were no longer tested for brief periods of time but were allowed to live in the experimental chambers. These chambers were equipped with three levers, one for self-stimulation, one for delivery of 90 mg food pellets and one for the delivery of 0.1 ml of water for each lever press. All three levers were maintained on continuous reinforcement schedule and were available at all times. The chambers were maintained in a 12 hr dark/light cycle with lights on from 7 AM to 7 PM. Temperatures were maintained at 20°C and a constant air flow of 4 liters/min was put through each chamber.

The behavior of the animals in these chambers was very different from the behavior of animals we described previously. Following a period of intense self-stimulation very similar to that seen in the short test situations, the animals began alternating periods of lever pressing on all three levers followed by rest periods (Fig. 10). This series of alternating behaviors was controlled by the diurnal cycle in that responding occurred most frequently during the dark (25).

Exposure of animals to 10% oxygen resulted in a prolonged period of intense self-stimulation lasting for approximately 12 hr, accompanied by an almost total cessation of food and water intake (Fig. 11). Following this 12 hr increased rate of responding,

same level as the control period during the end of the test session. At the higher level of exposure, there was a very significant decline in response rates in the normal animals that led to almost total cessation of lever pressing during the last 5 min of exposure on all three days (Fig. 9). In the dehydrated animals there was a 10-min delay before response rates declined, but rates subsequently decreased on all three days of exposure. During the three days of recovery following the trichloroethylene exposure, both groups of animals recovered towards control.

Thus it would appear that self-stimulation behavior is a sensitive indicator of the effects of organic solvents and it is of interest to note that during the more moderate exposure the animals showed a brief period of increased response rates suggesting a facilitative effect of this compound on self-stimulation behavior.
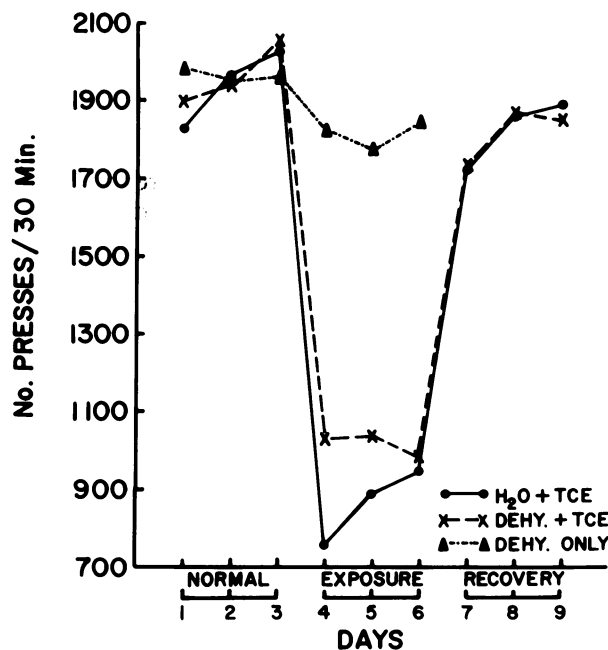

FIGURE 9. Mean lever-pressing rate of rats as a function of exposure to 3000 ppm of trichloroethylene. Dehydration condition was in effect only during the 3 days of exposure.
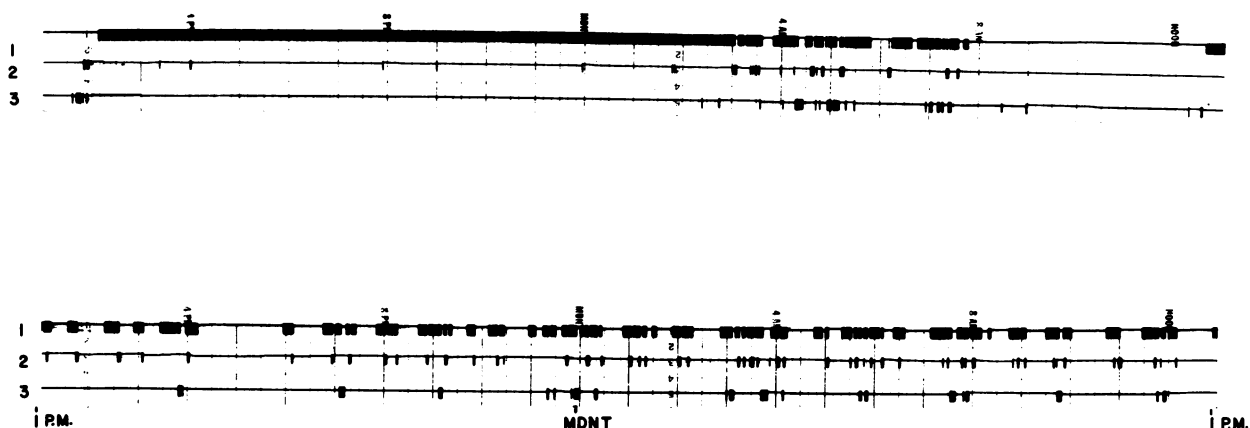
FIGURE 10. Illustration of typical self-stimulation, food and water intake pattern of a rat during the first two 24-hr periods in the three lever experimental chamber. Each downstroke of the recorder represents one response on: (1) the self-stimulation lever, (2) the food lever and, (3) the water reinforcement lever.
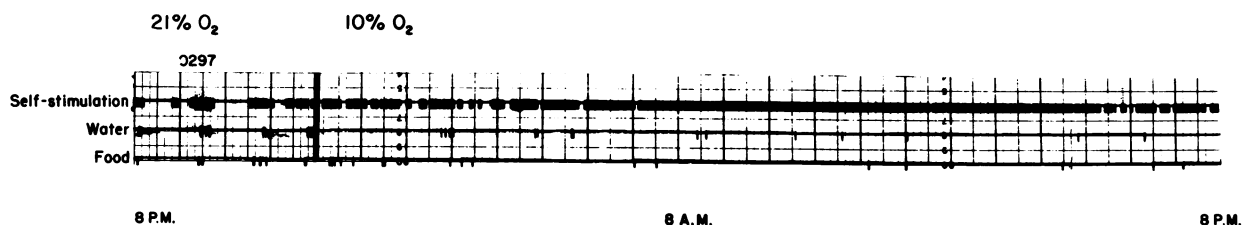

FIGURE 11. Transition from the periodic response pattern seen in 21% oxygen in a stable animal to the sustained self-stimulation and decreased response rate on the other two levers during the initial 12 hr in 10% oxygen.

the rate of self-stimulation declined to below control levels and during the following 36 hours of hypoxia tended to return towards control. Food and water intake showed some recovery but did not return to control rates during the entire 48-hr exposure period (26). We also showed that this was a dose-dependent effect in that during exposure to 14% oxygen the elevation in self-stimulation rates was not as marked as at 10%, and the reduction in food and water intake was also less (27).

This effect of hypoxia was very interesting to us for two reasons: first, the effect was very similar to what the administration of amphetamine produced on these behaviors, as shown by Stein (5). Second, the decreased food and water intake and the enhancement of self-stimulation were very suggestive of the anorexia and euphoria reported by mountineers at altitude. Since the administration of amphetamine has been shown to release catecholamines in the brain (6) we decided to investigate whether this also occurred during hypoxia.

The series of studies that followed this observation demonstrated that the enhanced self-stimulation response of the animal shown in 10% oxygen was not due to the release of peripheral catecholamines, since the blocking or elimination of

these by adrenal demedullation and peripheral administration of 6-OH-dopamine hydrobromide did not affect the response (28). Forebrain norepinephrine levels in animals exposed for 6, 12, and 24 hr to 10% oxygen however, in Figure 12, indicated that there was a significant decline in total norepinephrine content of the forebrain following 12 hr exposure. After 24 hr of exposure to 10% oxygen, norepinephrine content returned to control levels. Thus it appeared from our experiments that exposure of animals to hypoxia resulted in a predictable series of behavioral and neurochemical consequences.

In these experiments, we reported that when animals were exposed to 8% oxygen the self-stimulation rates declined instead of increasing. At that time we were unaware that ambient temperature was an important variable in determining the organism's response to hypoxia. Thus in our most recent observation, we have exposed two groups of animals to 8% oxygen in a three lever experimental chamber maintaining themselves on continuous reinforcement schedules. One group of animals was maintained at 30°C environment temperature and another group at 20°C. As can be seen from Figure 13, animals exposed to 8% oxygen at 20°C increased
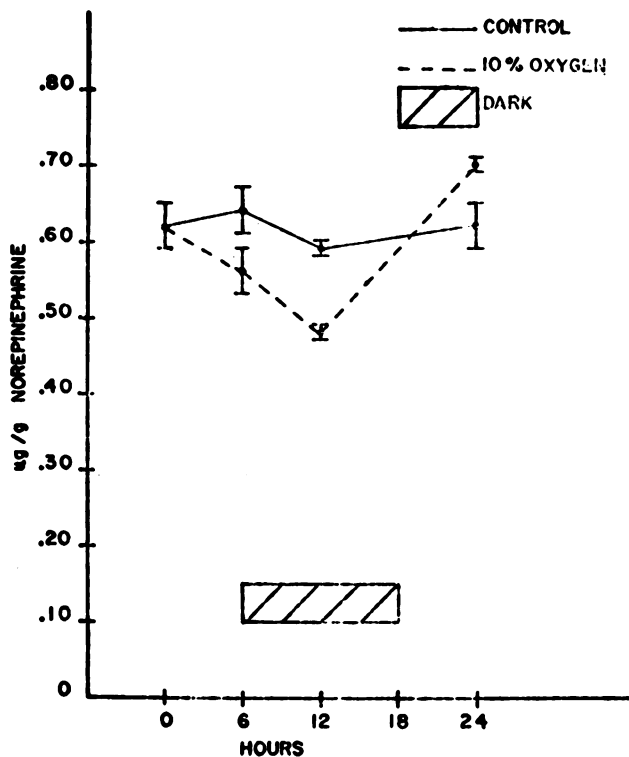
FIGURE 12. Forebrain norepinephrine levels of rats exposed to either 21% oxygen or 10% oxygen for 6, 12, or 24 hrs. The animals were in a 12-hr light/dark cycle with lights off from 6-12 hr.
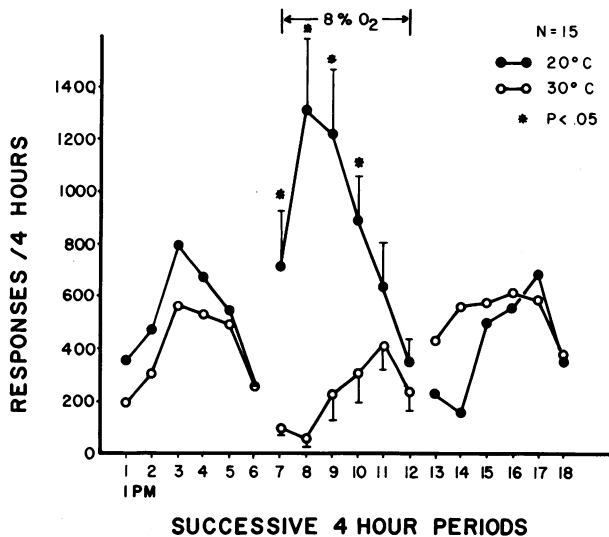

FIGURE 13. Three consecutive 12-hr periods of responding for self-stimulation in two groups of 15 rats, at two ambient temperatures. The animals were in 21% oxygen during the first 24 hr, in 8% oxygen during the next 24 hr, and in 21% oxygen during the last 24 hr.

their self-stimulation markedly for a 12-hr period with a subsequent steep decline. Animals exposed at 30°C on the other hand, showed profound depression of self-stimulation behavior for the first 12-hr period and then recovered towards control rate. During the following 24-hr period in 21% oxygen, they returned towards control rates after some apparent compensation. This compensation seemed to consist of higher than normal rates for the animals that were not active during the hypoxic exposure and unusually low rates for the animals that showed the increased self-stimulation. Food and water intake were not temperature dependent in that both behaviors were profoundly depressed by 8% oxygen, regardless of environmental temperature. This intriguing observation of the differential effects of environmental temperature on self-stimulation during hypoxia lead us to inquire as to the physiological concomitants of this exposure. In our next experiment, therefore, we measured the body temperature of animals exposed to hypoxia at various environmental temperatures (30). Thus it can be seen from Figure 14 that when animals were exposed to 8% oxygen and 0.1% carbon monoxide at 20°C there is a rapid decline in both brain and peritoneal temperatures as recorded with chroni-
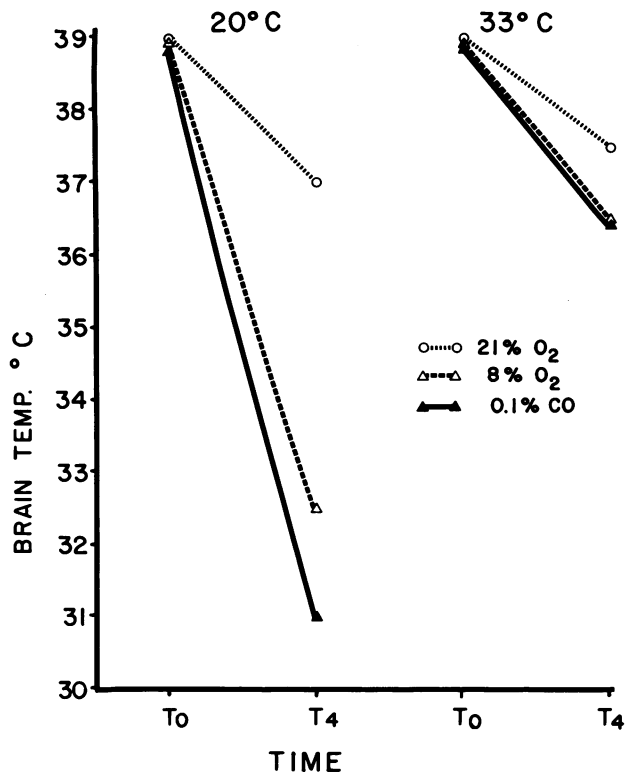

FIGURE 14. Alterations in brain temperature of two rats, each exposed to 0.1% carbon monoxide as well as 8% oxygen at either 20°C or 30°C for 4 hr.

cally implanted thermistors. During a period of 4 hr of exposure there is a decline of 7°C to 8°C in body temperature. These animals, although unrestrained and unanesthetized, were not self-stimulators, and therefore we are at present unable to say whether the body temperature of vigorously working animals undergoes this type of decline also. Other animals exposed to 8% oxygen at 30°C, however, show no decline in body temperature during a 4-hr exposure. In fact, from some other experiments in our laboratory as well as results reported by others, it appears that when environmental temperatures are at or above 30°C oxygen concentrations which are easily tolerated at lower temperatures can become lethal. Similar physiological changes occur in animals exposed equivalent concentrations of carbon monoxide.

Our data indicate that the self-stimulation technique can be used as a sensitive and quantitative index of the neurobiological effects of environmental agents. The use of this technique has enabled us to contrast the effects of acute exposures where behavioral decrements were observed in hypoxia with prolonged exposures where facilitation of self-stimulation occurred simultaneously with depression of food and water intake. This behavioral contrast may reflect the effect of hormonal influences on behavior observed only during prolonged periods of stress.

The importance of environmental temperatures in determining behavioral output during toxic exposures was also revealed by the use of self-stimulation technique. The complex relationships between behavior, body temperature, environmental temperatures as well as hormonal responses in the organism indicate that only by studying these interactions will we arrive at a clear understanding of the biological effects of environmental toxins.

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